REMARKS

Status of the Claims.

Claims 1-21 are pending with entry of this amendment, claims 22-67 being cancelled and no claims being added herein.

Election/Restriction.

Pursuant to a restriction requirement made final, Applicants cancel claims 22-67 with entry of this amendment. Please note, however, that Applicants reserve the right to file subsequent applications claiming the canceled subject matter and the claim cancellations should not be construed as abandonment or agreement with the Examiner's position in the Office Action.

35 U.S.C. §102.

Claims 1-7, 11, 12, 20, and 21 were rejected under 35 U.S.C. §102(e) as allegedly anticipated by Mathies *et al.* and under 35 U.S.C. §102(b) as allegedly anticipated by Mathies *et al.* (WO 00/42424). Applicants traverse.

The Examiner is respectfully reminded that anticipation requires that "**all limitations** of the claim are found in the reference, or 'fully met' by it." *Kalman v Kimberly-Clark Corp.,* 218 USPQ 781, 789 (Fed. Cir. 1983). In the instant case, claim 1 recites:

> 1.   A method of determining the sequence of a nucleic acid template, said method comprising:
>   i)   generating and redox labeling sets of complementary sequencing fragments of said template where the sets of fragments terminating with the four different bases A, C, G, or T are each label labeled with a redox-active label that has an oxidation state distinct and distinguishable from the redox states of the labels labeling the other sets of fragments;
>   ii)   separating said sequencing fragments;
>   iii)   performing cyclic voltammetry on said sequencing fragments to produce a cyclic voltammogram for the redox-labeled sequencing fragments;
>   iv)   detecting the signal for each redox-active label **at a phase angle out of phase with respect to the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label**.

Claim 1 thus provides a detection step where the redox active tags are selectively discriminated from each other by **nulling** their response (*i.e.*, **zeroing out the response**). The specific tag is detected by a **drop-out of signal**, rather than the presence of signal. As stated in the specification (and captured in the claim language):

> In preferred embodiments, **the discrimination of one tag versus all others is accomplished through a "phase-nulling" technique**. In this approach, the signal for each tag is **selectively eliminated** while the other three responses remain virtually unchanged. [emphasis added] (page 18, line 30, to 19, line 2)

> *   *   *

> At the frequency and phase angle producing the "phase-common" signal, a large signal is observed for all four tags, with typically minimal loss in signal compared to that observed at each of the optimum phase angles. **The phase-nulled signal for each redox-active tag can then be obtained at the same frequency (*e.g.*, third harmonic) by locking-in at a phase angle that is out of phase from the optimum for each tag**. The phase angle is preferably **either + or - 90 degrees out of phase**. **The signal corresponding to the phase-nulled component is effectively diminished while the other three tags are relatively unaffected**. When compared to the phase-common signal, it is easy to determine which peak has been removed. This method thus allows rapid "tag calling" on a single set of data simply by identifying the peaks that disappear.

This is in contrast to most phase-locked detection approaches that use selective filters **to enhance** the signal.

The Mathies *et al.* references simply fail to disclose or otherwise teach or suggest a detection approach that involves discriminating redox-active tags by phase nulling (dropping out of the signal.

To the contrary, rather than **altering phase angle** to drop out a the signal from a particular label as recited in the presently pending claims, the Mathies *et al.* references teach **altering the voltage of particular** electrodes and matrix coding the resulting signals:

> Due to the use of two oxidative and two reductive labels, **a matrix coding method is used to interpret these signals as illustrated in FIG. 3**. The signals at an oxidative electrode for a particular label are categorized as "positive high (+1) or low (0)", depending on whether a significant signal is

seen above the background at that potential. Reductive signals (which are of opposite polarity) are characterized as "negative high (or -1) and low (0)", again depending on whether significant signal is seen above the background at that potential. **The label with the higher oxidation potential (V2) is detected at only the most oxidative electrode, so it is given the matrix code of (0,1).** This is illustrated by label 1 in FIG. 3 that, under the particular conditions of this experiment, is oxidized at potential V2 but is not oxidized at the electrode poised at the lower potential V1. **The other oxidative label with a lower oxidation potential V1 is detected at both oxidative electrodes, therefore it is given the code (1,1). Analogously, the two reductive signals would be coded (0,-1) and (1,-1).** Consequently, each of the labels has a unique "signed binary code" or matrix value, and therefore can be unequivocally identified. **This coding method provides a way to decompose the detection signals from each other, thereby ensuring unique measurement of the electrochemical signals from the various labeled analytes.** [emphasis added] (US 6,361,671 col. 7, line 63, to col. 8, line 20 and WO 0042424 page 11, line 24, to page 12, line 9).)

The only mention whatsoever of phase locking in the Mathies *et al.* references pertains to methods to increase signal to noise ratio:

But, as the sensitivity of these voltammetric techniques is at most in the micromolar concentration range, they may not be sensitive enough to detect low levels of analytes such as DNA sequencing products. In that case, sinusoidal voltammetric detection can be utilized, as it is capable of better selectivity and sensitivity in comparison to the traditional voltammetric detection (Singhal, P., Kawagoe, K. T., Christian, C. N., and Kuhr, W. G., (1997) Anal. Chem. 69, 1662-1668). This technique uses a large amplitude sinusoidal waveform to scan across a potential window on an electrode surface. Instead of the traditional approach of looking at the electrochemical signal versus time, this technique relies on the harmonic isolation and **digital phase locking** of electrochemical signals. **It is at least two orders of magnitude more sensitive** than constant potential detection, and up to four orders of magnitude more sensitive than cyclic voltammetric detection. [emphasis added] (US 6,361,671, col. 9, lines 31-47, WO 00/42424, page 14, lines 1-7).

Again, the Mathies *et al.* references contemplate the use of phase locking to **increase** the signal to noise ratio. There is no disclosure whatsoever regarding a detection method that involves ". . . detecting the signal for each redox-active label **at a phase angle out of phase** with respect to the

optimum phase angle for said redox-active label, **where a drop-out of signal** at said phase angle **indicates the presence** of said redox-active label."

The Mathies *et al.* references thus fail to provide all limitations of the presently pending claims and the rejections under 35 U.S.C. §102(b) and §102(e) should be withdrawn.

35 U.S.C. §103(a).

Claims 8 and 9 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Mathies *et al.* (WO 00/42424) in view of Ihara *et al.* (1996) *Nucleic Acids Res.*, 24(21): 4273-4280. Claim 10 was rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Mathies *et al.* (WO 00/42424) in view of Nishino *et al.* (1996) *J. Organic Chem.*, 61: 7534-7544. Claims 12-19 were rejected under 35 U.S.C. §103(a) as allegedly obvious in light of Mathies *et al.* (WO 00/42424) in view of Kuhr *et al.* (U.S. 5,650,061). Applicants traverse.

A *prima facie* case of obviousness requires that the combination of the cited art, taken with general knowledge in the field, **must provide all of the elements of the claimed invention**. When a rejection depends on a combination of prior art references, there must be some teaching, suggestion, or motivation to combine the references. *In re Geiger*, 815 2 USPQ2d 1276, 1278 (Fed. Cir. 1987). Moreover, to support an obviousness rejection, the cited references must additionally provide a reasonable expectation of success. *In re Vaeck*, 20 USPQ2d 1438 (Fed. Cir. 1991), *citing In re Dow Chemical Co.*, 5 USPQ2d 1529, 1531 (Fed. Cir. 1988). The cited art fails to provide all the elements of the claimed invention and, in fact, teaches away from the presently claimed invention.

In the instant case, as explained above, the pending claims are directed to a method of determining the sequence of a nucleic acid template, where the method involves a detecting step comprising:

> . . . detecting the signal for each redox-active label **at a phase angle out of phase with respect to the optimum phase angle for said redox-active label, where a drop-out of signal at said phase angle indicates the presence of said redox-active label**.

As explained above, Mathies *et al.* (WO 00/42424) fails to teach or suggest such a method and, in fact, actually **teaches away from such a detecting step**. Rather than **altering phase angle** to drop out a the signal from a particular label, as recited in the presently pending claims, the

Mathies *et al.* reference teaches **altering the voltage of particular** electrodes and matrix coding the resulting signals to discriminate analytes. Moreover, as explained above, Mathies *et al.* contemplates the use of digital phase locking of electrochemical signals to maximize sensitivity, **not to drop out a particular signal**. Mathies *et al.* thus clearly teaches away from the presently claimed invention.

This defect of Mathies *et al.* **is not** remedied by the remaining cited references. Ihara *et al.*, for example, teaches the making of ferrocene oligonucleotide conjugates for "probing" DNA. This reference simply describes the use of cyclic voltammetry to determine the half potentials (E1/2, of the ferrocene-labeled probe under various solution conditions (*see, page* 4276, col. 1). This reference offers no teaching or suggestion of a detection method that involves ". . . detecting the signal for each redox-active label **at a phase angle out of phase with respect to the optimum phase angle** for said redox-active label, where a **drop-out** of signal at said phase angle indicates the presence of said redox-active label."

Nishino *et al.* simply discloses the synthesis of linear amphipathic porphyrin dimmers and trimers and porphyrin arrays. This reference offers no teaching or suggest regarding any voltammetric methods whatsoever.

Kuhr *et al.* discloses a cyclic voltammetry method and system in which a large amplitude sine wave is applied as the excitation potential to a amperometric measurement to produce a current output that is a phase shifted sine wave containing faradaic information at many frequencies. A current obtained from a conventional potentiostat coupled to the electrode is coupled to a lock-in amplifier that monitors the signal at one frequency at a specified phase angle at a higher harmonic of the fundamental frequency of the sinusoidal sweep. By monitoring the higher harmonic components, the faradaic signal is therefore distinguished from the background signal thereby improving sensitivity and signal to noise ration.

This reference, however, offers no teaching or suggestion of a detection method that involves detection **at a phase angle out of phase with respect to the optimum phase angle to effect a drop out of the desired signal**. To the contrary, this reference teaches away from the presently claimed method by teaching detection of the signal at the optimum phase angle:

> The step of selectively detecting the voltammetric response comprises detecting a current from the analyte **at a selected phase angle of**

> **measurement to enhance a detected signal corresponding to the redox**
> **species of interest**. [emphasis added] (col. 4, lines 13-17)

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> The amplitude and frequency of the excitation waveform is determined by the
> electrochemical properties of the analyte as well as the background signal, **so**
> **as to maximize the signal due to the analyte at higher harmonics** while
> minimizing the contribution to the background currents in the same frequency
> regime. Detection frequency and phase angle are determined by examining
> the frequency spectrum of the analyte and **finding the harmonic at which**
> **the analyte has the highest signal-to-noise ratio**. The **phase angle is**
> **determined by maximizing the signal magnitude** while minimizing the
> contributions due to the background. [emphasis added] (col. 4, lines 50-60)

Kuhr *et al.* thus expressly teaches away from the presently claimed method. The combination of the cited references thus expressly teaches away from the presently claimed method. Accordingly the Examiner has failed to make his *prima facie* case and the rejections under 35 U.S.C. §103(a) should be withdrawn.

Obviousness-Type Double Patenting.

Claims 1-21 were rejected under the judicially created doctrine of obviousness-type double patenting in light of claims 26, 29, and 30 of Mathies *et al.* (U.S. 6,361,671). Applicants traverse.

As explained above, the presently pending claims are directed to a method of determining the sequence of a nucleic acid template, where the method involves a detecting step comprising:

> . . . detecting the signal for each redox-active label **at a phase angle out of**
> **phase with respect to the optimum phase angle for said redox-active**
> **label, where a drop-out of signal at said phase angle indicates the**
> **presence of said redox-active label**.

Claims 26, 29, and 30 of Mathies *et al.* offer no teaching or suggestion of a detection step that involves detecting a signal **at a phase angle out of phase with respect to the optimum phase angle** for said redox-active label, to effect a signal **drop-out**. To the contrary, claim 26 simply provides for simultaneously detecting the distinct electrochemical signals generated by the redox labels

to identify the individual fragments", while claims 29 and 30 provide for detecting the different electrochemical signals by sinusoidal voltammetric detection of electrochemical signals at **a plurality of electrodes**. In this regard, it is noted that the plurality of electrodes was required by Mathies *et al.* to facilitate the matrix coding scheme to distinguish the electrodes (*see, e.g.,* discussion above, also Mathies *et al.* col. 7, line 63, to col. 8, line 20). Claims 26, 29, and 30 simply fail to teach or suggest the presently claimed method and the remainder of this patent expressly teaches away. Accordingly, the obviousness-type double patenting rejection should be withdrawn.

In view of the foregoing, Applicants believe all claims now pending in this application are in condition for allowance. The issuance of a formal Notice of Allowance at an early date is respectfully requested. Should the Examiner seek to maintain the rejections, Applicants request a telephone interview with the Examiner and the Examiner's supervisor.

If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at (510) 769-3513.

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Respectfully submitted,

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